HHS Public Access

Author manuscript

AIDS. Author manuscript; available in PMC 2016 June 22.

Published in final edited form as:

AIDS. 2015 February 20; 29(4): 433–442. doi:10.1097/QAD.0000000000000560.

Cerebral white matter integrity during primary HIV infection

Patrick W. Wright[a,b], **Florin F. Vaida**[c], **Ricardo J. Fernández**[d], **Jerrel Rutlin**[e], **Richard W. Price**[f], **Evelyn Lee**[f], **Julia Peterson**[f], **Dietmar Fuchs**[g], **Joshua S. Shimony**[h], **Kevin R. Robertson**[i], **Rudolph Walter**[j], **Dieter J. Meyerhoff**[j,k], **Serena Spudich**[l,*], and **Beau M. Ances**[a,b,*]

[a]Department of Biomedical Engineering, School of Medicine, St. Louis, Missouri

[b]Department of Neurology, School of Medicine, St. Louis, Missouri

[c]Department of Family and Preventive Medicine, UCSD, San Diego, California, USA

[d]Department of Biology, University of Puerto Rico, Río Piedras, San Juan, Puerto Rico

[e]Department of Psychiatry, WUSTL School of Medicine, St. Louis, Missouri

[f]Department of Neurology, UCSF, San Francisco, California, USA

[g]Division of Biological Chemistry, Innsbruck Medical University, Innsbruck, Austria

[h]Department of Radiology, WUSTL School of Medicine, St. Louis, Missouri

[i]Department of Neurology, UNC, Chapel Hill, North Carolina

[j]Center for Imaging of Neurodegenerative Diseases, VA Medical Center, San Francisco

[k]Department of Radiology and Biomedical Imaging, UCSF, San Francisco, California

[l]Department of Neurology, Yale University, New Haven, Connecticut, USA

Abstract

Objective—Inflammation and infection within the central nervous system is initiated during primary HIV infection (PHI), but the association of these processes with the integrity of brain white matter during PHI is unknown.

Design—We used diffusion tensor imaging (DTI) in this prospective cross-sectional neuroimaging study to determine the extent of white matter involvement in early HIV infection.

Methods—Antiretroviral-naive PHI (defined as <1 year after infection, $n = 62$), chronic HIV infection (CHI, $n = 16$), and HIV-uninfected ($n = 19$) participants had DTI, laboratory, and neuropsychometric performance assessments. DTI metrics were examined using region of interest and whole brain voxelwise analyses. Linear mixed-effects models assessed correlations between DTI measures and laboratory and neuropsychometric performance values.

Correspondence to Beau M. Ances MD, PhD, Box 8111, 660 South Euclid Ave, Saint Louis, MO 63110, USA., Tel: +1 314 747 8423; fax: +1 314 747 8427; bances@wustl.edu.

*Drs Serena S. Spudich and Beau M. Ances contributed equally to the writing of this article.

Conflicts of interest

There are no conflicts of interest in this study.

Results—PHI participants were assessed at a median 4.1 months after estimated infection, and had median $CD4^+$ cell count of 573 cells/μl, and HIV-1 RNA viral load of 4.5 $\log_{10}$ copies/ml in plasma and 2.6 $\log_{10}$ copies/ml in cerebrospinal fluid (CSF). DTI metrics in PHI individuals were similar to HIV— participants and correlated with disruptions in the blood-brain barrier (indicated by CSF/plasma albumin ratio and CSF protein). CHI participants had significant loss of white matter integrity that correlated with biomarkers of infection and inflammation (blood viral load, $CD4^+$ T-cell count, and neopterin, and CSF white blood cell). Within the PHI group, DTI metrics inversely correlated with increasing days since infection.

Conclusion—In individuals assessed during PHI, group DTI measures suggested relative preservation of white matter microstructural integrity, but were associated with disruption of the blood-brain barrier and estimated duration of infection.

Keywords

corpus callosum; cerebrospinal fluid/plasma albumin ratio; diffusion tensor imaging; neopterin; neuropsychometric testing; primary HIV infection; white matter

Introduction

HIV enters the central nervous system (CNS) soon after initial infection in the form of free virions or by way of immune cells travelling across the blood–brain barrier (BBB). Once in the brain, HIV targets perivascular macrophages [1] stimulating immune activation. Markers of systemic and microglia immune activation are elevated in cerebrospinal fluid (CSF) during primary HIV infection (PHI; defined here as ≤1 year after exposure), and remain elevated with chronic HIV infection (CHI) (>1 year) [2,3]. Viral and immunopathogenic changes that occur during PHI may provide the substrate for subsequent development of HIV-associated neurocognitive disorders, including breakdown of the BBB and inflammatory changes that facilitate subsequent neurodegeneration in CHI [4]. However, the stage at which these alterations occur in the brain due to HIV infection remains poorly characterized.

Diffusion tensor imaging (DTI) is a noninvasive MRI technique that can be used to assess white matter integrity by quantifying the diffusion of water in the brain [5]. Commonly quantified DTI metrics (used here as an umbrella term to include multiple calculated measures) are mean diffusivity, the average diffusion in all directions, and fractional anisotropy which is a normalized scalar quantity that ranges from zero to one. Higher fractional anisotropy values indicate diffusion predominately parallel to axons, likely restricted by intact myelin [6]. The corpus callosum, a prominent, homogeneous dense white matter tract containing interhemispheric cortical connections, among other white matter tracts, has been extensively examined in HIV-infected patients using DTI [7–15]. Most commonly, white matter integrity is affected, with HIV-infected individuals having low fractional anisotropy and high mean diffusivity when compared with HIV-uninfected controls. However, some DTI studies in HIV patients also have shown increases in fractional anisotropy [16], an effect that could be due to a loss of transverse fibers in a voxel, leaving only parallel tracts to influence anisotropy. Abnormalities in DTI metrics can be improved

after the initiation of combination antiretroviral therapy (cART) [12]. To date, most DTI studies only have focused on CHI individuals receiving cART.

Changes that can occur in the brain during PHI, including altered white matter integrity, have not been well characterized. The few neuroimaging studies that have been performed in PHI individuals demonstrated low cerebral blood flow [13], neuroinflammation [14,15,17], and reduced brain volume [18]. In this study, we obtained DTI, laboratory measures, and neuropsychometric performance from HIV-infected and HIV-uninfected individuals. We primarily focused on PHI individuals but included a small cohort of CHI participants to probe our methods' consistency with known DTI characteristics seen with prolonged HIV infection. A region of interest (ROI) approach targeting the corpus callosum was employed along with an exploratory voxelwise analysis to assess for potential widespread white matter changes. DTI metrics were also correlated with laboratory measures and common neuropsychometric performance measures. We hypothesized that DTI measures within the corpus callosum are affected in PHI and these metrics correlate with measures of BBB breakdown and inflammation.

Materials and methods

Participants

This study included PHI ($n = 62$), CHI ($n = 16$), and HIV-uninfected ($n = 19$) participants. PHI individuals were assessed within 1 year of acquiring HIV as confirmed by a recent negative result on HIV antibody testing or ELISA [19]. The date of HIV exposure was estimated as 14 days before onset of seroconversion symptoms [20] or as the date halfway between the last negative and first positive HIV test [21]. Duration of infection for CHI participants was determined by date of first positive HIV test and/or recalled date of seroconversion. PHI participants were naive to cART, whereas CHI patients were either naive to cART ($n = 9$) or had elected to stop therapy for at least 3 months before entering this study ($n = 7$, approximate mean time off therapy: 11.7 months). HIV-associated neurocognitive disorder (HAND) classification was not determined as only a relatively brief testing battery was employed and no measures of functional impairment were obtained. HIV-uninfected participants without preexisting medical conditions and with similar age, social, and demographic factors to the PHI individuals were recruited from the community; they underwent CSF collection for study purposes only. The institutional review board at the University of California San Francisco approved the protocol and informed consent was obtained from all participants.

Specimen sampling, processing, and laboratory studies

Clinical examination (including medical and neurological) and laboratory studies (CSF and blood) were obtained before neuroimaging [22]. Details of the laboratory analysis have been described previously [3]. Blood $CD4^+$ T-lymphocyte counts were measured using flow cytometry. Paired blood and CSF samples were analyzed for white blood cell (WBC) count, protein, and albumin. Paired cell-free CSF and blood plasma were analyzed for neopterin concentrations and HIV RNA viral load. For all comparisons done in this study, clinical and laboratory values were grouped into three categories: those representing HIV viral burden

($CD4^+$ T-cell count, plasma viral load, CSF viral load), BBB integrity (CSF protein, CSF/plasma albumin ratio), and inflammation (plasma neopterin, CSF WBC count, CSF neopterin). Although blood TNF-α and interleukin-6 are important markers of systemic processes during primary HIV infection, CSF levels of these markers have not been clearly shown to predict the development of HIV-associated neurocognitive disorders (HAND). Thus, these biomarkers were not examined, as variables for this study focused on CNS outcomes in HIV CSF monocyte chemoattractant protein-1 (MCP-1), a measure of monocyte/macrophage infiltration in the CNS, was collected in this cohort but the total numbers of individuals with both DTI scans and CSF MCP-1 measures were too small to pursue in additional analyses for the current study.

Neuropsychometric performance evaluation

A neuropsychometric performance battery, including timed gait, grooved pegboard, finger tapping, and digit-symbol tasks, was administered to participants. All tests, excluding timed gait, were standardized according to normative data for age, education, sex, and ethnicity and converted to *z*-scores that were then averaged to calculate a composite neuropsychological summary *z*-score (NPZ-4).

Neuroimaging acquisition

MRI was performed at the Center for Imaging of Neurodegenerative Diseases of the San Francisco Veterans Administration Medical Center using a 4T Siemens/ Bruker MedSpec whole-body scanner equipped with a Siemens TRIO console (Siemens AG, Erlangen, Germany) and a product transmit/receive 8-channel head coil. A T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE, TR/T1/TE = 2300/950/3 ms, voxel size = 1.0 × 1.0 × 1.0 mm^3, flip angle 7°, bandwidth = 200Hz/ pixel) was acquired. Four consecutive DTI scans were acquired for each participant to increase signal-to-noise (TR/TE = 6000/77 ms, 2.0 × 2.0 × 3.0 mm^3 voxels, flip angle 90°, six directions, b-values = 0 and 800s/mm^2).

Diffusion tensor imaging processing and region of interest analysis

Details of image registration, motion correction, and diffusion tensor estimation for DTI metric calculation have been described previously [23,24]. The entire corpus callosum was delineated semiautomatically on five midsagittal slices from the fractional anisotropy map of each participant using Analyze (Mayo Clinic, Rochester, Minnesota, USA) with a fractional anisotropy threshold of 0.3 in an attempt to limit partial volume effects. The sampled corpus callosum volume was segmented into five vertical partitions [25] using MATLAB (Mathworks, Natick, Massachusetts, USA). These corpus callosum subregions correspond to partitions containing inter-hemispheric connections for prefrontal [corpus callosum 1 (CC1)], premotor and supplementary motor (CC2), primary motor (CC3), primary sensory (CC4), and parietal, temporal, and occipital cortices (CC5).

Voxelwise whole brain analysis of diffusion tensor imaging measures

A voxelwise analysis was performed using the Tract-Based Spatial Statistics (TBSS) package (FMRIB, University of Oxford, Oxford, UK) [26,27]. After correcting all images

for eddy currents, fractional anisotropy maps were computed using the FMRIB diffusion toolbox and aligned to a standard image space using the FMRIB nonlinear image registration tool [28]. Calculated DTI metric images were projected onto a mean, group-dependent fractional anisotropy skeleton (thresholded at fractional anisotropy = 0.3) for voxelwise analyses. Group comparisons were performed using FMRIB Randomise, a statistical approach that corrects for multiple comparisons using permutation testing [29]. Ten thousand permutations were used to estimate the null distributions of each comparison, as this number provides a margin of error for $P = 0.05$ of less than 9% ($P = 0.05 \pm 0.0044$). Age was covaried within each group in all correlation analyses.

Statistical analysis

Demographic characteristics were summarized for the three groups using means and standard deviations for the continuous variables and compared among groups using the F-test of analysis of variance. Mean diffusivity and fractional anisotropy values were log-transformed or analyzed on the square (power = 2) scale, respectively, to improve normality. The three groups were compared with respect to their ROI-specific DTI measures using a linear mixed-effects model with a subject-specific random effect and including corpus callosum region as a factor. No correction was performed for multiple comparisons for the three groups because each pairwise comparison corresponded to different hypotheses done at the $P = 0.05$ level. Additional analyses adjusted for age. However, these analyses should be interpreted with caution because older age correlates with CHI categorization in this study, and is arguably a mediator of the effect of chronic infection.

The association of DTI metrics with laboratory measures of HIV infection, NPZ-4 score, and duration of infection was examined via Pearson's correlation and simple linear regression, separately for the PHI and CHI groups, for each of the five corpus callosum regions. The analyses used a Holm-Bonferroni correction for multiple comparisons involving the five corpus callosum regions.

Results

Participants

Demographic data are shown in Table 1. PHI individuals were assessed at an estimated mean 4.1 (2.5 SD) months after initial HIV infection. No significant differences existed among PHI, CHI, and HIV-uninfected participants with regard to sex, education, and NPZ-4 score, plasma viral load, neopterin. $CD4^+$ T-cell count, age, and all CSF measures differed among groups (all $P < 0.05$).

Diffusion tensor imaging metrics of white matter integrity are affected in chronic HIV infection but not in early primary HIV infection

We focused our initial attention on the corpus callosum as this region is a prominent white matter tract responsible for interhemispheric communication and has been shown to be affected by HIV [6,8–10,30]. In general, PHI and HIV-uninfected groups had comparable values, whereas CHI patients had substantial differences in DTI metrics across all corpus callosum subregions compared with the other groups. For mean diffusivity and fractional

anisotropy, pairwise comparisons across corpus callosum subregions demonstrated no significant differences between HIV-uninfected and PHI groups (mean diffusivity: $P = 0.59$, fractional anisotropy: $P = 0.84$), but both metrics were higher in CHI compared with PHI and HIV-uninfected (mean diffusivity: both $P < 0.05$, fractional anisotropy: both $P < 0.001$) (Fig. 1a and b). These relationships were maintained throughout cerebral white matter using whole brain voxelwise comparisons (Fig. 1c and d).

Correlations between diffusion tensor imaging metrics and laboratory measures of HIV infection in primary HIV infection and chronic HIV infection

The correlations of fractional anisotropy and mean diffusivity with laboratory markers for PHI and CHI from all corpus callosum regions are tabulated in Supplemental Digital Content 1 (Table http://links.lww.com/QAD/A622). In brief, for the PHI participants, DTI metrics in CC2 and CC3 showed correlations with markers typically associated with disruption of the BBB (e.g., CSF/plasma albumin ratio and CSF protein) and plasma viral load, but not measures of inflammation. The albumin ratio and CSF protein measures were also highly correlated (Spearman's $\rho = 0.93$), consistent with their shared putative roles as biomarkers of BBB integrity. NPZ-4 performance did not correlate with DTI metrics from any corpus callosum region in the PHI group (Fig. 2a – f).

DTI metrics in CHI correlated with markers of HIV viral burden (e.g. blood $CD4^+$ T-cell count and viral load) as well as indicators of cellular activation (e.g. plasma neopterin and CSF WBC), but not BBB integrity, using an ROI analysis. In the CHI group, NPZ-4 performance correlated with DTI metrics in CC2 ($P = 0.004$), CC3 ($P = 0.017$), and CC4 ($P = 0.031$) (Fig. 2). These regions in particular contain tracts connecting motor regions that are likely the most implicated in our psychomotor NPZ-4 battery, providing further validation of the sensitivity of our corpus callosum segmentation and sampling methods. Data for CC1 and CC5 are not shown because they were not significantly implicated using our neuropsychological battery.

Voxelwise comparisons further supported the relationships between DTI metrics and laboratory values observed in the ROI analysis, with the CHI group showing more pervasive effects (Fig. 3a and b). Moreover, mean diffusivity from CHI participants showed correlations with both plasma viral load and CSF viral load (data not shown). For PHI individuals, fractional anisotropy in the body of the corpus callosum correlated with plasma viral load (data not shown) and $CD4^+$ T-cell count correlated with fractional anisotropy in the anterior half of the corpus callosum. In the CHI group, fractional anisotropy in the anterior white matter tracts correlated with plasma viral load. Although no significant correlations were observed in the corpus callosum ROI analysis between CHI and CSF/protein albumin ratio, other white matter regions showed a relationship. It is possible that duration of infection dependence exists within this affected location. Importantly, the voxelwise analysis showed a strong correlation between both mean diffusivity and fractional anisotropy with plasma neopterin in the anterior portion of the corpus callosum of CHI participants. This effect was also supported by our ROI analysis, providing additional agreement between the two methods. Significant relationships were based on correlations in expected directions (e.g. CHI fractional anisotropy and viral load were inversely correlated).

Diffusion tensor imaging metrics correlate with duration of HIV infection in the corpus callosum

We also analyzed HIV duration of infection as a function of DTI metrics. Within the PHI group alone, a significant relationship between fractional anisotropy and duration of infection was seen exclusively within CC1 (semipartial correlation $R = -0.284$ (controlling for age, $P = 0.028$) (Fig. 4a and b). When the PHI and CHI participants were merged into a single group, an effect of duration of infection (plotted on a $\log_2$ scale) was observed for mean diffusivity [semipartial correlation $R = 0.350$ (controlling for age), $P = 0.002$] and fractional anisotropy (semipartial correlation $R = -0.426$ (controlling for age), $P \leq 0.001$) (Fig. 4a and b). Only fractional anisotropy and mean diffusivity from CC1 showed such a strong relationship with duration of infection (data not shown for CC2–CC5). These results imply that there is a continuous effect of time detectable within the range of durations of infection in this cohort.

Discussion

We show that white matter integrity as measured by DTI is not altered in a group of PHI participants (mean: 4.1 months after infection). However, white matter integrity is significantly altered in an untreated CHI group (mean: 11.3 years since diagnosis). Our results in this small CHI cohort are similar to previous reports of treated and untreated CHI participants that demonstrated a reduction in fractional anisotropy and an increase in mean diffusivity [12]. In our study, observed changes in DTI metrics correlated more strongly with laboratory values for CHI rather than PHI participants. When looking at the continuum of HIV infection, a negative correlation was observed between DTI metrics and increasing duration of infection, even within the first year of infection. Our findings suggest that HIV is associated with changes in white matter integrity soon after infection and these changes become more readily apparent with prolonged infection. These results also suggest the potential utility of DTI to assess HIV-related disorder noninvasively.

Comparisons of DTI metrics with CSF and blood biomarkers may begin to provide us a timeline for mechanisms of white matter injury initiated during early HIV infection. Within the PHI group, markers of BBB breakdown (CSF/plasma albumin ratio and CSF protein) correlated with DTI metrics, but it is interesting that we actually did not observe any significant differences in DTI metrics between the large group of PHI participants and the HIV-uninfected participants, despite the previously (well characterized) observed effects in CHI using DTI [8–14]. However, when we analyzed with respect to duration of infection, we did observe changes in fractional anisotropy relatively early after seroconversion. It may be that arbitrary grouping using a cutoff of less than 1 year duration of HIV may miss progressive changes. It is possible that the observed results reflect the existence of a biphasic model of white matter degradation in which alterations are due to different HIV-dependent mechanisms depending on the current stage of infection. Specifically, during PHI, increased BBB permeability may drive observed white matter changes, whereas the mechanisms underlying continued alterations observed at later times shift from a dependence on BBB integrity to ongoing chronic cell-mediated inflammation. Larger group sizes and longitudinal analyses are needed to support this hypothesized model.

Within the small CHI cohort, markers of inflammation (neopterin and immune cells in the blood and CSF, respectively) correlated with DTI metrics. Activation of both infected and uninfected macrophages and microglia in the brain could propagate inflammation and edema and influence DTI measures. In particular, a greater abundance of inflammatory cells seen in CHI, as well as surrounding debris, could affect the free path of water diffusion and lead to changes in both fractional anisotropy and mean diffusivity [31,32]. Cloak *et al.* [33] showed a strong correlation between frontal white matter mean diffusivity and myoinositol, a glial metabolite. These findings imply that increased diffusion may reflect increased glial activation or inflammation. We observed significantly higher radial diffusivity in CHI than both PHI and HIV-uninfected controls (data not shown). This effect may reflect inflammatory vasogenic edema rather than localized myelin degradation [34,35].

This study has several limitations. The focus of this study, unlike many others, was to study potential early changes in white matter brain integrity of PHI. Relatively fewer numbers of CHI and HIV-uninfected participants were recruited compared with PHI individuals, thus reducing statistical power to detect significant differences between groups. Because of the cross-sectional nature of this study, we are unable to comment on the within-participant effect of HIV progression over time. Future studies that assess PHI participants longitudinally are needed to fully characterize the effects of HIV infection on cerebral white matter during this time and to subsequently determine the optimal time for initiating cART (as we chose to examine the effects of HIV itself without influence of therapy). Furthermore, duration since diagnosis in the CHI participants was obtained by self-report and has potential recall bias and might be distant from actual time of HIV acquisition. We considered the laboratory-confirmed PHI disease duration to be critical to the analysis, whereas the less precise estimated CHI durations were valuable even if they underestimated actual length of infection. Also, the ROI and TBSS methods were largely in agreement, but differences may arise because of the different processing streams used as well as the relatively narrow volume sampled from the corpus callosum in the ROI analysis. Although mean age differed between HIV-infected groups (PHI: 36.9 years old, CHI: 45.1 years old), previous studies of DTI changes in healthy aging have reported significant age-dependent alterations in time windows usually spanning a minimum of 20 years [36]. We did not consider the effects of other potential comorbidities (e.g. alcohol and methamphetamine abuse, smoking, hepatitis coinfection, etc.) that have been implicated in changes in brain structure and function in HIV-infected individuals. Although outside of the scope of this study, it will be important to examine the impact of these factors in PHI. Finally, although the correlation coefficients for the significant associations observed between callosal DTI metrics and duration of infection and clinical measures within the PHI group were less than 0.5, we believe they are critical to report as they may point to the mechanisms of early HIV pathophysiology in the brain.

Conclusion

In summary, DTI metrics that reflect white matter integrity were not abnormal in this study of early PHI, but they correlated with markers of BBB breakdown. Moreover, fractional anisotropy decreased as a function of duration of infection within the first year of infection. In contrast, DTI metrics from CHI participants correlated strongly with clinical markers of

inflammation and disease progression. The strong relationship between fractional anisotropy from the PHI group and duration of infection existing at the cross-sectional level supports pursuing longitudinal analysis to elucidate further the relationship between duration of HIV infection and its neurodegenerative capacity. These data provide further evidence of the utility of DTI metrics as noninvasive biomarkers of white matter change in HIV infection, and they emphasize the importance of diagnosing HIV soon after infection to possibly preempt further neuropathology through early treatment.

Acknowledgments

The authors sincerely thank the study participants who enrolled in this project and the staff at the UCSF Options Project and Magnet, as well as Liz Westerhaus, MA at Washington University in Saint Louis for administrative assistance.

Study concept and design: B.M.A., D.J.M., R.W.P., S.S.S., P.W.W.; acquisition of data: E.L., D.J.M., J.P., R.W.P., S.S.S., R.W.; analysis and interpretation of data: B.M.A., R.J.F., D.F., R.W.P., K.R.R., J.R., J.S.S., S.S.S., F.F.V., P.W.W.; drafting of the manuscript: B.M.A., S.S.S., F.F.V., P.W.W.; critical revision of the manuscript for important intellectual content: all authors; statistical analysis: F.F.V., P.W.W.; administrative, technical, or material support: E.L., J.P., R.W.; study supervision: B.M.A., S.S.S.

Source of funding: Design and conduct of the study, collection, management, analysis and interpretation of the data were supported by the National Institute of Mental Health [R01 MH081772, R21 MH099979, K23 MH074466, K23 MH081786], National Institute of Allergy and Infectious Diseases [P01 A1071713, M01 RR00083]; National Institute of Nursing Research [R01 NR012907, R01 NR014449, and R01 NR012657] UCSF AIDS Research Institute, UCSF Academic Senate, UCSF REAC, and the Radiology Research Service at the SF VA Medical Center. Funding organizations had no role in preparation, review, or approval of the manuscript or the decision to submit the manuscript for publication.

References

1. Ances BM, Ellis RJ. Dementia and neurocognitive disorders due to HIV-1 infection. Semin Neurol. 2007; 27:86–92. [PubMed: 17226745]
2. Hagberg L, Cinque P, Gisslen M, Brew BJ, Spudich S, Bestetti A, et al. Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. AIDS Res Ther. 2010; 7:15. [PubMed: 20525234]
3. Spudich S, Gisslen M, Hagberg L, Lee E, Liegler T, Brew B, et al. Central nervous system immune activation characterizes primary human immunodeficiency virus 1 infection even in participants with minimal cerebrospinal fluid viral burden. J Infect Dis. 2011; 204:753–760. [PubMed: 21844301]
4. Spudich S, Gonzalez-Scarano F. HIV-1-related central nervous system disease: current issues in pathogenesis, diagnosis, and treatment. Cold Spring Harb Perspect Med. 2012; 2:a007120. [PubMed: 22675662]
5. Le Bihan D, Johansen-Berg H. Diffusion MRI at 25: exploring brain tissue structure and function. Neuroimage. 2012; 61:324–341. [PubMed: 22120012]
6. Chanraud S, Zahr N, Sullivan EV, Pfefferbaum A. MR diffusion tensor imaging: a window into white matter integrity of the working brain. Neuropsychol Rev. 2010; 20:209–225. [PubMed: 20422451]
7. Thurnher MM, Castillo M, Stadler A, Rieger A, Schmid B, Sundgren PC. Diffusion-tensor MR imaging of the brain in human immunodeficiency virus-positive patients. AJNR Am J Neuroradiol. 2005; 26:2275–2281. [PubMed: 16219833]
8. Filippi CG, Ulug AM, Ryan E, Ferrando SJ, van Gorp W. Diffusion tensor imaging of patients with HIV and normal-appearing white matter on MR images of the brain. AJNR Am J Neuroradiol. 2001; 22:277–283. [PubMed: 11156769]

9. Muller-Oehring EM, Schulte T, Rosenbloom MJ, Pfefferbaum A, Sullivan EV. Callosal degradation in HIV-1 infection predicts hierarchical perception: a DTI study. Neuropsychologia. 2010; 48:1133–1143. [PubMed: 20018201]
10. Wu Y, Storey P, Cohen BA, Epstein LG, Edelman RR, Ragin AB. Diffusion alterations in corpus callosum of patients with HIV. AJNR Am J Neuroradiol. 2006; 27:656–660. [PubMed: 16552012]
11. Pfefferbaum A, Rosenbloom MJ, Rohlfing T, Kemper CA, Deresinski S, Sullivan EV. Frontostriatal fiber bundle compromise in HIV infection without dementia. AIDS. 2009; 23:1977–1985. [PubMed: 19730350]
12. Wright PW, Heaps JM, Shimony JS, Thomas JB, Ances BM. The effects of HIV and combination antiretroviral therapy on white matter integrity. AIDS. 2012; 26:1501–1508. [PubMed: 22546990]
13. Ances BM, Sisti D, Vaida F, Liang CL, Leontiev O, Perthen JE, et al. Resting cerebral blood flow: a potential biomarker of the effects of HIV in the brain. Neurology. 2009; 73:702–708. [PubMed: 19720977]
14. Lentz MR, Kim WK, Kim H, Soulas C, Lee V, Venna N, et al. Alterations in brain metabolism during the first year of HIV infection. J Neurovirol. 2011; 17:220–229. [PubMed: 21494901]
15. Valcour V, Chalermchai T, Sailasuta N, Marovich M, Lerdlum S, Suttichom D, et al. Central nervous system viral invasion and inflammation during acute HIV infection. J Infect Dis. 2012; 206:275–282. [PubMed: 22551810]
16. Stebbins GT, Smith CA, Bartt RE, Kessler HA, Adeyemi OM, Martin E, et al. HIV-associated alterations in normal-appearing white matter: a voxel-wise diffusion tensor imaging study. J Acquir Immune Defic Syndr. 2007; 46:564–573. [PubMed: 18193498]
17. Peluso MJ, Meyerhoff DJ, Price RW, Peterson J, Lee E, Young AC, et al. Cerebrospinal fluid and neuroimaging biomarker abnormalities suggest early neurological injury in a subset of individuals during primary HIV infection. J Infect Dis. 2013; 207:1703–1712. [PubMed: 23460748]
18. Ragin AB, Du H, Ochs R, Wu Y, Sammet CL, Shoukry A, et al. Structural brain alterations can be detected early in HIV infection. Neurology. 2012; 79:2328–2334. [PubMed: 23197750]
19. Zetola NM, Pilcher CD. Diagnosis and management of acute HIV infection. Infect Dis Clin North Am. 2007; 21:19–48vii. [PubMed: 17502228]
20. Lindback S, Thorstensson R, Karlsson AC, von Sydow M, Flamholc L, Blaxhult A, et al. Diagnosis of primary HIV-1 infection, duration of follow-up after HIV exposure Karolinska Institute Primary HIV Infection Study Group. AIDS. 2000; 14:2333–2339. [PubMed: 11089621]
21. Little SJ, Frost SD, Wong JK, Smith DM, Pond SL, Ignacio CC, et al. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. J Virol. 2008; 82:5510–5518. [PubMed: 18353964]
22. Spudich SS, Nilsson AC, Lollo ND, Liegler TJ, Petropoulos CJ, Deeks SG, et al. Cerebrospinal fluid HIV infection and pleocytosis: relation to systemic infection and antiretroviral treatment. BMC Infect Dis. 2005; 5:98. [PubMed: 16266436]
23. Shimony JS, Burton H, Epstein AA, McLaren DG, Sun SW, Snyder AZ. Diffusion tensor imaging reveals white matter reorganization in early blind humans. Cereb Cortex. 2006; 16:1653–1661. [PubMed: 16400157]
24. Shimony JS, Sheline YI, D'Angelo G, Epstein AA, Benzinger TL, Mintun MA, et al. Diffuse microstructural abnormalities of normal-appearing white matter in late life depression: a diffusion tensor imaging study. Biol Psychiatry. 2009; 66:245–252. [PubMed: 19375071]
25. Hofer S, Frahm J. Topography of the human corpus callosum revisited: comprehensive fiber tractography using diffusion tensor magnetic resonance imaging. Neuroimage. 2006; 32:989–994. [PubMed: 16854598]
26. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: voxelwise analysis of multisubject diffusion data. Neuroimage. 2006; 31:1487–1505. [PubMed: 16624579]
27. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 2004; 23(Suppl 1):S208–S219. [PubMed: 15501092]

28. Rueckert D, Sonoda LI, Hayes C, Hill DL, Leach MO, Hawkes DJ. Nonrigid registration using free-form deformations: application to breast MR images. IEEE Trans Med Imaging. 1999; 18:712–721. [PubMed: 10534053]

29. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp. 2002; 15:1–25. [PubMed: 11747097]

30. Tate DF, Sampat M, Harezlak J, Fiecas M, Hogan J, Dewey J, et al. Regional areas and widths of the midsagittal corpus callosum among HIV-infected patients on stable antiretroviral therapies. J Neurovirol. 2011; 17:368–379. [PubMed: 21556960]

31. Anderson AW, Xie J, Pizzonia J, Bronen RA, Spencer DD, Gore JC. Effects of cell volume fraction changes on apparent diffusion in human cells. Magn Reson Imaging. 2000; 18:689–695. [PubMed: 10930778]

32. Wang Y, Wang Q, Haldar JP, Yeh FC, Xie M, Sun P, et al. Quantification of increased cellularity during inflammatory demyelination. Brain. 2011; 134:3590–3601. [PubMed: 22171354]

33. Cloak CC, Chang L, Ernst T. Increased frontal white matter diffusion is associated with glial metabolites and psychomotor slowing in HIV. J Neuroimmunol. 2004; 157:147–152. [PubMed: 15579292]

34. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage. 2002; 17:1429–1436. [PubMed: 12414282]

35. Lodygensky GA, West T, Stump M, Holtzman DM, Inder TE, Neil JJ. In vivo MRI analysis of an inflammatory injury in the developing brain. Brain Behav Immun. 2010; 24:759–767. [PubMed: 19945527]

36. Bennett IJ, Madden DJ. Disconnected aging: cerebral white matter integrity and age-related differences in cognition. Neuroscience. 2013; 276:187–205. [PubMed: 24280637]

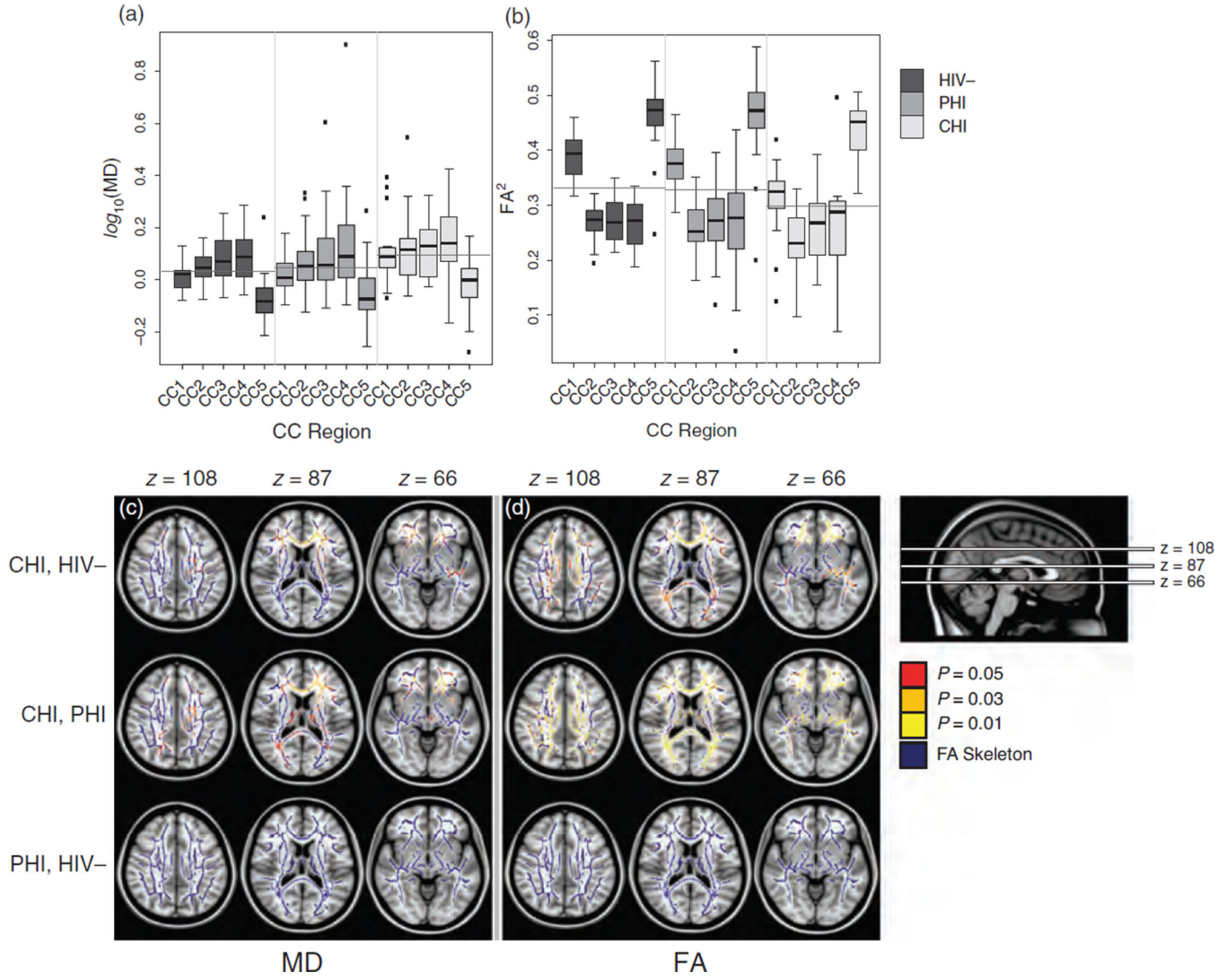

Fig. 1. Groupwise region of interest and voxelwise comparisons of mean diffusivity and fractional anisotropy

Five vertical partitions from the corpus callosum were sampled using an ROI analysis for both mean diffusivity (a) and fractional anisotropy (b). CHI participants had substantial differences in DTI metrics compared with other groups, whereas PHI and HIV-uninfected (HIV-) had comparable values. Horizontal line: group mean. Whiskers extend to either 1.5Q below Q1 or 1.5Q above Q3. (c) Voxelwise comparisons of mean diffusivity for (starting from top): CHI>HIV–, CHI>PHI, PHI>HIV–. (d) Comparisons of fractional anisotropy for (starting from top): CHI<HIV– CHI<PHI, PHI<HIV–. Only CHI, not PHI, showed significant changes in mean diffusivity and fractional anisotropy relative to HIV-uninfected participants in the anterior forceps, optic radiations, and other white matter tracts. Red: P=0.05; Orange: P= 0.03; Yellow: P= 0.01. CC, corpus callosum; CHI, chronic HIV infection; FA, fractional anisotropy; MD, mean diffusivity; PHI, primary HIV infection.

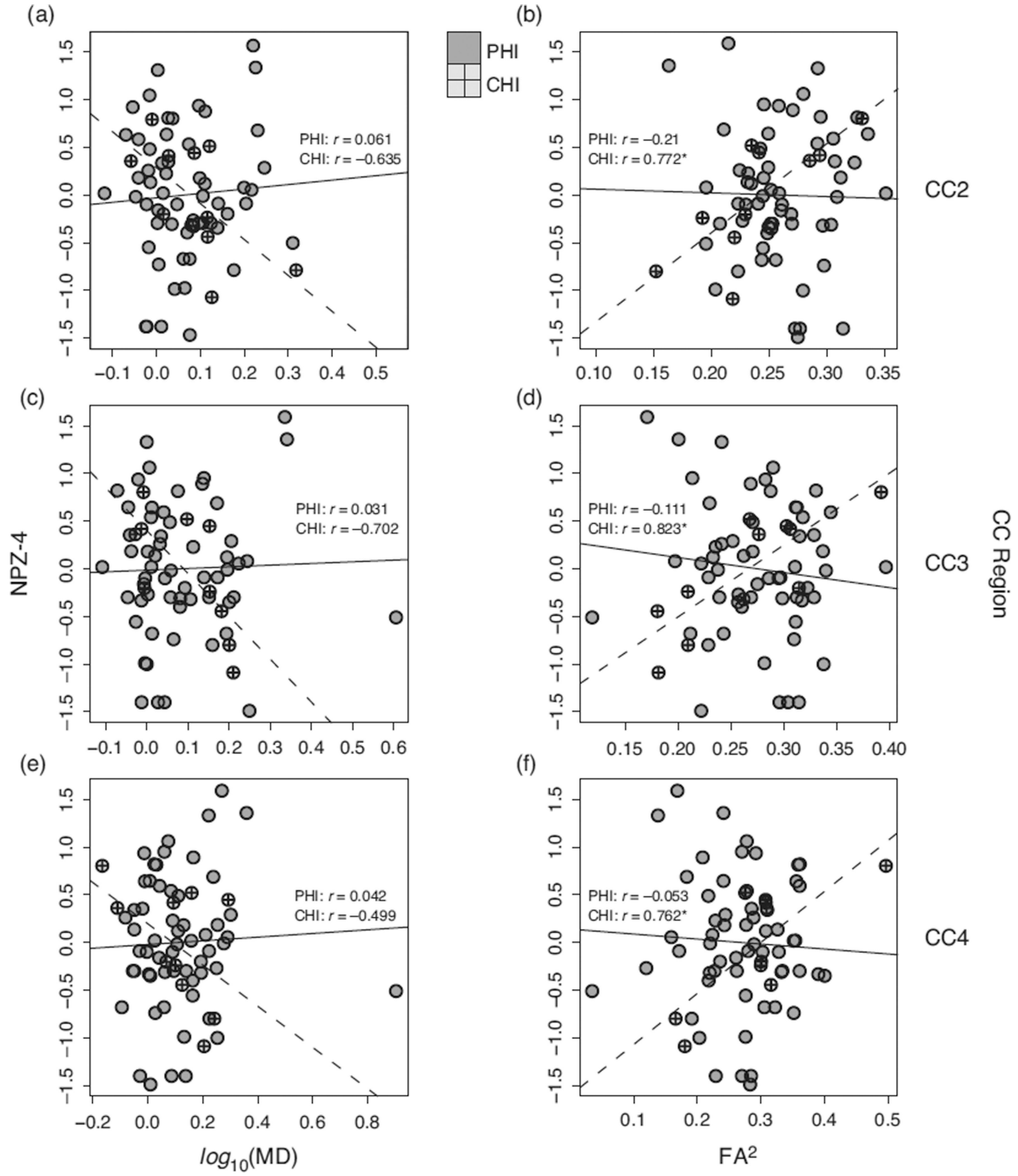

Fig. 2. Relationship between fractional anisotropy and mean diffusivity and neuropsychological performance, average *z*- score from four tests

(a and b) Mean diffusivity and fractional anisotropy from CC2 correlated with NPZ-4. (c and d) Mean diffusivity and fractional anisotropy from CC3 correlated with NPZ-4. (e and f) Mean diffusivity and fractional anisotropy from CC4 correlated with NPZ-4. *$P < 0.05$. Fractional anisotropy from CC2-CC4 from the CHI group significantly correlated with neuropsychometric performance, the regions expected to be implicated by our psychomotor battery. NPZ-4 data were missing from four PHI and six CHI participants. Solid line: Best-fit line for PHI group. Dashed line: Best-fit line for CHI group. CC2-4, corpus callosum 2–4;

CHI, chronic HIV infection; FA, fractional anisotropy; MD, mean diffusivity; NPZ-4, neuropsychological performance, average *z*-score from four tests; PHI, primary HIV infection.

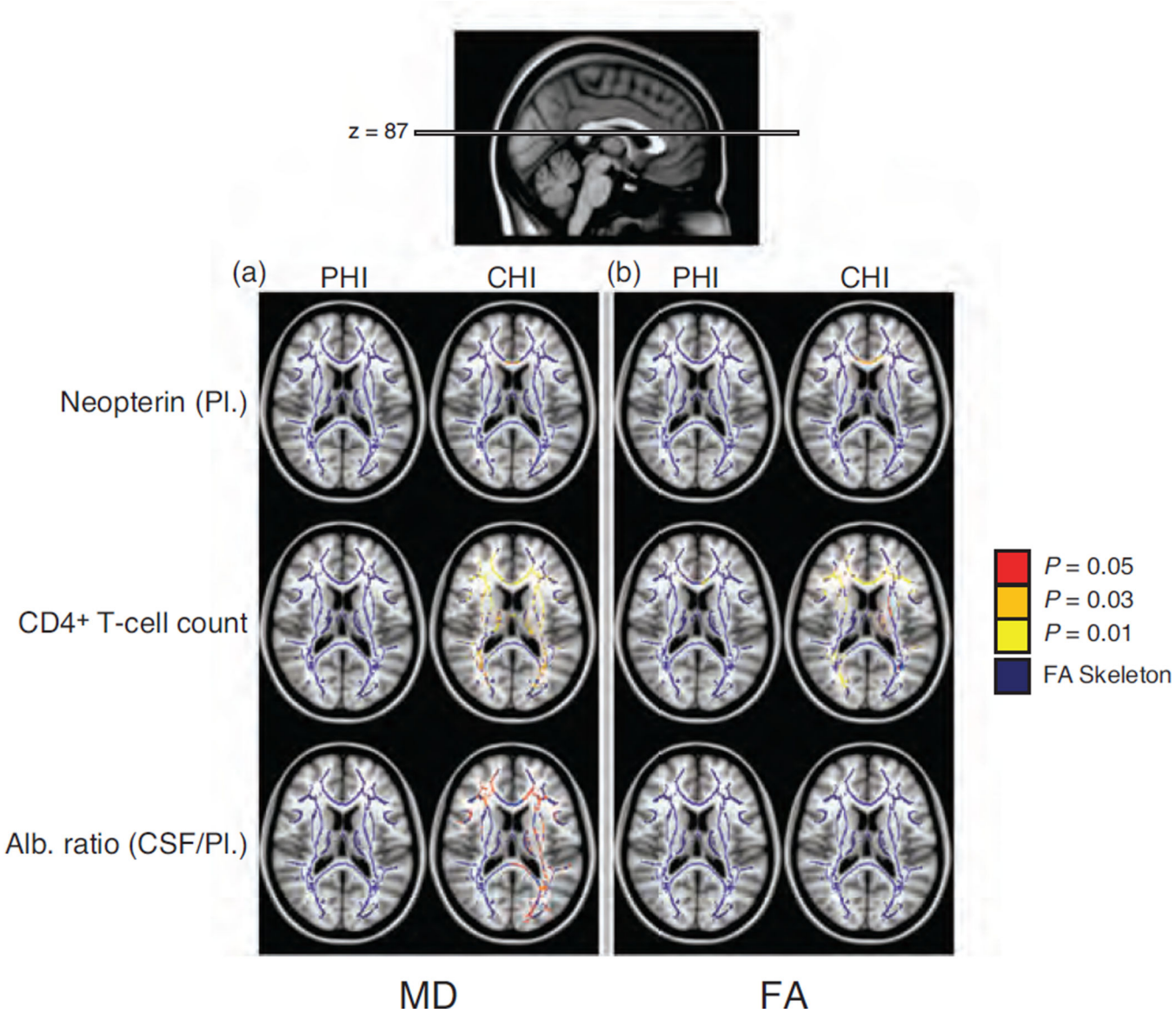

Fig. 3. Voxelwise correlations between mean diffusivity (a) and fractional anisotropy (b) from primary HIV infection and chronic HIV infection with plasma neopterin, $CD4^+$ T-cell count, and cerebrospinal fluid/plasma albumin ratio

CHI patients showed pervasive correlations with laboratory measures. White matter changes in the PHI group correlated with $CD4^+$ T-cell count (b, second row) and plasma viral load (data not shown). Red: $P = 0.05$; Orange: $P = 0.03$; Yellow: $P = 0.01$. CHI, chronic HIV infection; CSF, cerebrospinal fluid; FA, fractional anisotropy; MD, mean diffusivity; PHI, primary HIV infection.

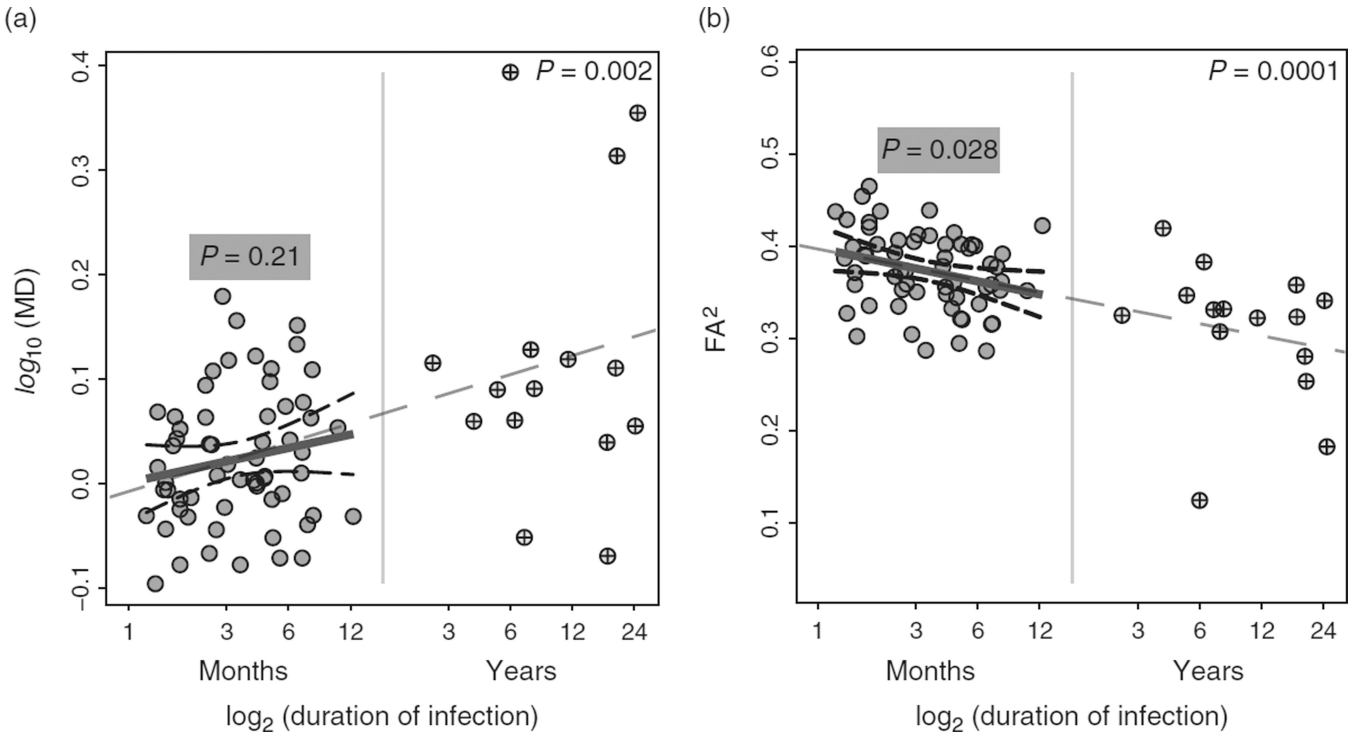

Fig. 4. Effect of duration of infection on mean diffusivity (a) and fractional anisotropy (b) in CC1

Both HIV-infected groups (PHI and CHI) are plotted as function of duration of infection. The time dependence (*x*-axis) is linear on the $\log_2$ scale. The regression line, 95% confidence intervals, and *P* value are included for the PHI group (solid line and shaded *P* value) and a regression line is included for the combined HIV-infected group (dashed line and top right *P* value). Both fractional anisotropy from the PHI group as well as both fractional anisotropy and mean diffusivity from a combined HIV-infected group (from CC1) showed significant correlation with duration of infection. CC1, corpus callosum 1; CHI, chronic HIV infection; FA, fractional anisotropy; MD, mean diffusivity; PHI, primary HIV infection.

Table 1

Demographic and clinical information for the study participants.

	HIV−	PHI	CHI	*P*[a]
Sample size (*n*)	19	62	16	
Age (years)	34.5 (9.9)	36.9 (9.1)	45.1 (10.3)	0.003
Sex (men/*n*)	19/19	62/62	15/16	0.17
Education (years)	16.1 (2.6)	15.4 (2.2)	14.3 (2.3)	0.083
Duration of infection[b] (months)		4.1 (2.5)	138.1 (96.9)	<0.001
NPZ-4	−0.4 (0.9)	0.0 (0.7)	0.0 (0.6)	0.21
$CD4^+$ T-cell count	790 (745, 1003)	573 (410, 748)	223 (145, 310)	<0.001
Plasma HIV-1 viral load (log_{10})		4.5 (3.9, 5.0)	4.6 (4.2, 5.0)	0.43
CSF HIV-1 viral load (log_{10})		2.6 (1.7,3.1)	4.1 (3.2,4.4)	<0.001
Plasma neopterin		17.1 (16.4)	20.6 (13.6)	0.55
CSF neopterin		12.9 (12.4)	36.7 (27.9)	<0.001
CSF white blood cell count	1.8 (1.5)	8.5 (9.9)	8.3 (6.8)	0.033
CSF protein	36.6 (8.8)	41.1 (13.3)	59.8 (18.9)	<0.001
CSF/Plasma albumin ratio	5.3 (1.9)	5.5 (2.3)	7.6 (3.5)	0.013

Mean (SD) or median (Q1, Q3) is shown. CHI, chronic HIV infection; CSF, cerebrospinal fluid; NPZ-4, neuropsychological performance, average *z*-score from 4 tests; PHI, primary HIV infection.

[a] *P* values reported for parameters possessed by all three groups are determined using analysis of variance for comparison across all groups.

[b] For PHI, duration of infection was estimated by date of recent seroconversion as confirmed by laboratory measures. CHI duration of infection reflected the period since known HIV diagnosis.